

A Method for Improving the Optical Properties of a Fluoregenic Di-metal Chelator as a Zn²⁺ Ion Fluorescent Probe by Using a Bridging Substrate

Min Sik Eom, Han Saem Park, Sudeok Kim, Kyungkyu Baek, Sang Yeob Ahn, Seok Keun Choi,[†] and Min Su Han^{*}

*Department of Chemistry, Chung-Ang University, Seoul 156-756, Korea. *E-mail: mshan@cau.ac.kr*

[†]Department of Civil Engineering, Chungbuk National University, Cheongju-si, Chungbuk 361-763, Korea

Received December 26, 2012, Accepted February 27, 2013

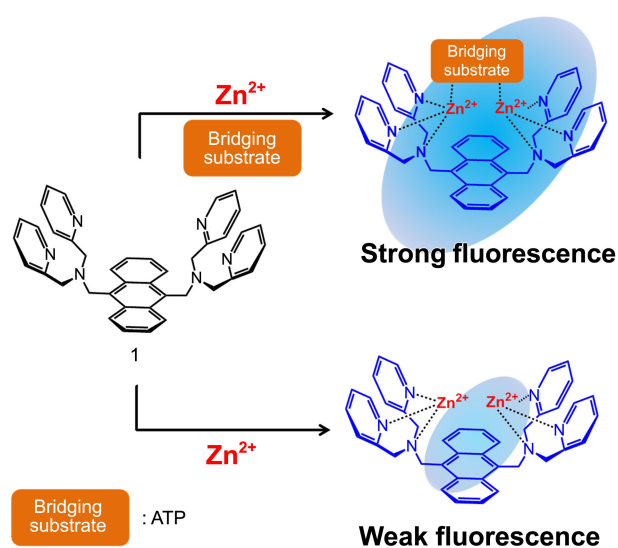
Key Words : Di-metallic complex, Bridging substrate, Zn²⁺, Adenosine triphosphate

Fluorescent probe based detection methods for metal ions are indispensable tools in many fields, including medical diagnostics, environmental monitoring, living cell studies, and electronics. These methods have multiple advantages over other methods, such as high sensitivity, low cost, ease of application, and versatility.¹ Numerous fluorescent metal ion probes have been designed using many strategies, including fluorogenic metal chelators, fluorescent dye tagged oligonucleotides, catalytic signal amplification, and chemodosimeters.^{1,2} Fluorogenic metal chelators, which consist of a fluorogenic unit (signaling site) covalently linked to chelating moieties (receptor units) with an appropriate spacer, are a general type metal ion probe. The recognition of chelating moieties with metal ions induces a change in the photophysical properties of the fluorescent probe. This is converted into an optical signal expressed as an enhancement or quenching of the fluorophore emission. The recognition can be enhanced to utilize additive reagents that provide additional binding sites for metal ions.³ For example, 8-aminoquinolino- β -cyclodextrin, developed by Liu *et al.*, exhibited cooperative binding to Zn²⁺ ion with 1-adamantanolic acid. It also detected Zn²⁺ ions more efficiently than without 1-adamantanolic acid because 1-adamantanolic acid bound to β -cyclodextrin to provide additional binding site for Zn²⁺ ions.^{3a} This strategy can easily expand to improve the optical properties of metal ion sensors with two metal binding sites. Di-metal complexes may include bridging substrates to complete the metal coordination sphere, and bridging substrates can modulate the properties of the resulting cascade complexes.⁴ In particular, bridging substrates provided additional metal ion binding sites and enhanced the binding properties of metal ions to metal ion ligands.⁵

Various fluorescent molecules with two metal binding sites have been synthesized as efficient probes for detecting metal ions, such as Zn²⁺ and Ag⁺.⁶ Scaffolds of di-metallic fluorogenic probes have also been devised to detect anions.⁷ Most of these molecules can be used as metal ion probes. The optical properties of these molecules can be improved by bridging substrates, which provide additional metal ion binding sites. To validate this concept, (9,10-bis[(2,2-dipicolylamino)methyl]anthracene)²⁺ (**1**) was adapted as a Zn²⁺ ion probe because [Zn₂(**1**)]⁴⁺ efficiently detects phosphate derivatives as potential bridging substrates to provide addi-

tional metal binding sites.⁸ In various phosphate derivatives, ATP was adapted as a bridging substrate to provide additional metal binding sites because [Zn₂(**1**)]⁴⁺ fluorescence is most enhanced by binding with ATP.⁸ Therefore, a combination of **1** and ATP (**1**/ATP) was prepared as a Zn²⁺ fluorescent probe. The optical properties of **1**/ATP, compared to those of **1**, can be improved by providing additional binding sites for metal ions using ATP, as depicted in Scheme 1.

To determine the optimal ratio of **1** and ATP, fluorescence titration of ATP was conducted using a 5 μ M solution of **1** with 2 equivalents of Zn²⁺ ions. The fluorescence emission spectra of [Zn₂(**1**)]⁴⁺ in the presence of varied ATP concentrations were evaluated. [Zn₂(**1**)]⁴⁺ fluorescence increased nearly proportionally to ATP concentration and was maximized at one equivalent of ATP (see Supporting information). A sensing system (**1**/ATP) consisting of **1** (5 μ M) and ATP (5 μ M) was prepared to evaluate the cooperative binding effect of the bridging substrate by providing additional binding sites for Zn²⁺ ions. Fluorescence changes of **1**/ATP in the presence of various concentrations of Zn²⁺ were examined. The addition of Zn²⁺ ions induced greatly enhanced fluorescence. The binding zinc ions within the bis-(2-picolyl)amine in **1** prevents the photo induced electron transfer process



Scheme 1. Schematic illustration of Zn²⁺ sensing system using bridging substrate.

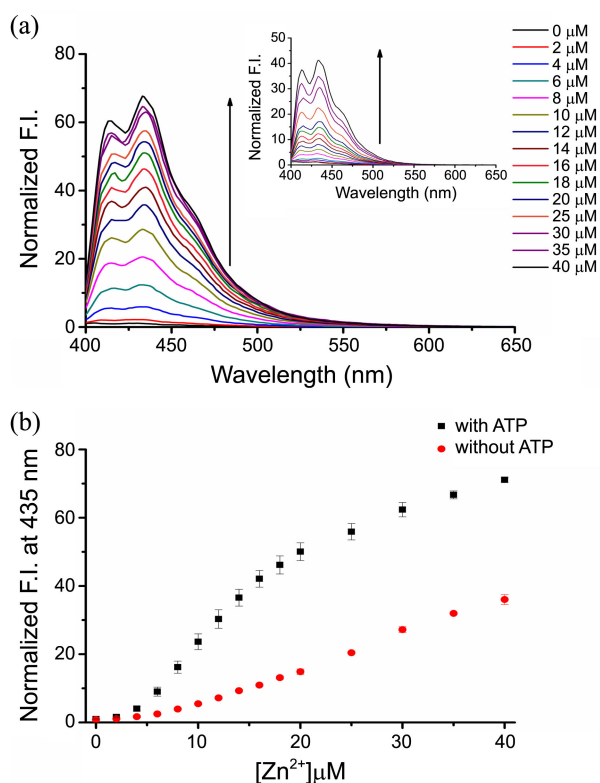


Figure 1. (a) Fluorescence emission spectra obtained by adding Zn^{2+} (0–40 μM) to pH 7.0 buffer solution (10 mM HEPES, $\text{H}_2\text{O}:\text{THF} = 3:1$) containing **1** (5 μM) with ATP (5 μM). Inset: without ATP. (b) Plot of normalized fluorescence intensities of **1** with ATP (■) and without ATP (●) at 435 nm.

from tertiary amine to anthracene, which induced the enhancement of fluorescence.⁹ In the presence of 10 μM of Zn^{2+} ions, the fluorescence intensity of the mixture was 3.5 times larger than the fluorescence intensity of **1** alone, as shown in Figure 1. Titration results were used to estimate the Zn^{2+} detection limits of the new sensing system and **1** alone as 4.5 μM and 15.6 μM , respectively (see Supporting Information). These results implied that ATP enhanced the binding affinity of **1** to Zn^{2+} by providing cooperative binding sites, which resulted in improved sensitivity of **1** in detecting Zn^{2+} ions.

ATP also enhanced the selectivity of **1** toward Zn^{2+} ion over other metal ions, including Cd^{2+} . To evaluate the role of ATP in the Zn^{2+} selectivity of the sensing system, fluorescence changes in **1** caused by other metal ions were measured in the presence or absence of ATP, as shown in Figure 2. The sensing system gave a negative response to other metal ions and a positive response to Cd^{2+} ion in both cases. However, the selectivity of **1** for Zn^{2+} over Cd^{2+} in the presence of ATP was 4.3 times higher than in the absence of ATP. This result showed that ATP enhances the selectivity of **1** for Zn^{2+} over other metal ions by providing selective binding sites for Zn^{2+} . This implies that the selectivity of metal ion probes with two metal binding sites can be modulated by a bridging substrate.

In general, pH plays a crucial role in anion recognition

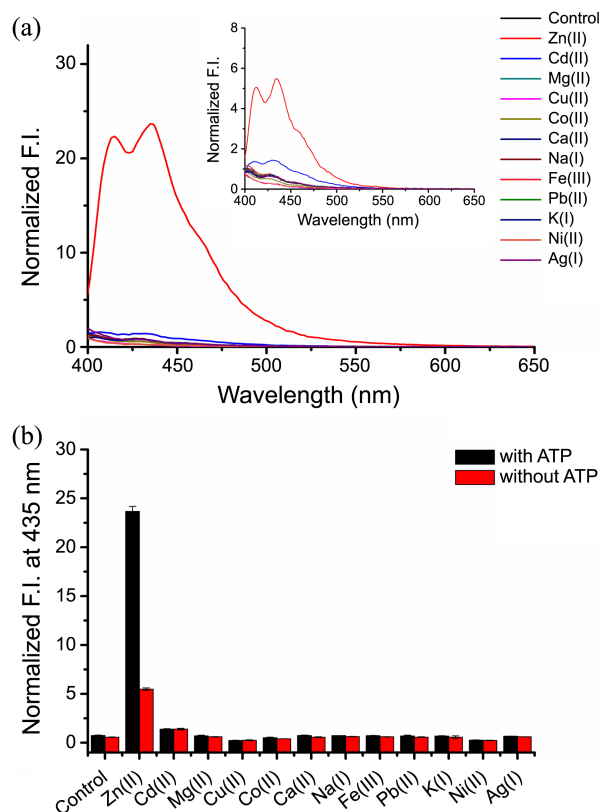


Figure 2. (a) Fluorescence spectra of **1** (5 μM) with ATP (5 μM) in the presence of various metal ions (10 μM). Inset: without ATP. (b) Plot of normalized fluorescence intensities of **1** with ATP (black) and without ATP (red) at 435 nm versus metal ions.

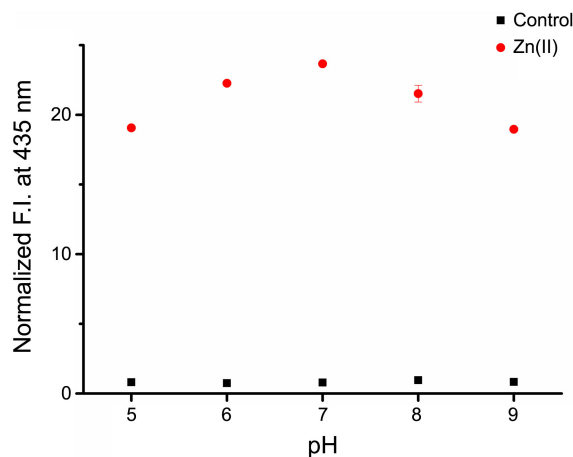


Figure 3. Fluorescence intensity of **1** (5 μM) with ATP (5 μM) at 435 nm in the presence and absence of Zn^{2+} (10 μM) at various pH values.

because anions may become protonated or deprotonated in certain pH windows. Therefore, pH values may affect the efficiency of ATP on the Zn^{2+} sensing ability of **1**. The effect of pH on the Zn^{2+} sensing ability of **1** in the presence of ATP was studied in a pH range of 5.0–9.0. As shown in Figure 3, the efficiency of ATP on the Zn^{2+} sensing is not sensitive to the pH values of assay mediums.

In conclusion, we developed a new strategy for improving

the sensing properties of fluoregenic molecules with two metal binding sites as metal ion sensors by using a bridging substrate. To validate this strategy, we evaluated various sensing properties of **1** for Zn²⁺ ion in the presence and absence of ATP. The sensitivity and selectivity of **1** toward Zn²⁺ were improved by providing additional binding sites via incorporating ATP as a bridging substrate. This approach might be applicable for improving other fluorogenic sensing systems.

Acknowledgments. This research was supported by the Chung-Ang University Research Grants in 2012.

References

- (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (b) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551. (c) Jiang, P.; Guo, Z. *Coord. Chem. Rev.* **2004**, *248*, 205. (d) Rurack, K. *Spectrochim. Acta A* **2001**, *57*, 2161.
- (a) Navani, N. K.; Li, Y. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272. (b) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386. (c) Wu, Q.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 14682. (d) Kim, M. H.; Jang, H. H.; Yi, S.; Chang, S.-K.; Han, M. S. *Chem. Commun.* **2009**, 4838. (e) Kaur, K.; Saini, R.; Kumar, A.; Luxami, V.; Kaur, N.; Singh, P.; Kumar, S. *Coord. Chem. Rev.* **2012**, *256*, 1992.
- (a) Chen, Y.; Han, K.-Y.; Liu, Y. *Bioorg. Med. Chem.* **2007**, *15*, 4537. (b) Zhang, Y.-M.; Chen, Y.; Li, Z.-Q.; Li, N.; Liu, Y. *Bioorg. Med. Chem.* **2010**, *18*, 1415.
- (a) He, H.; Martell, A. E.; Motekaitis, R. J.; Reibenspies, J. H. *Inorg. Chem.* **2000**, *39*, 1586. (b) Lu, Q.; Reibenspies, J. H.; Carroll, R. I.; Martell, A. E.; Clearfield, A. *Inorg. Chim. Acta* **1998**, *270*, 207. (c) Pauwels, T. F.; Lippens, W.; Smet, P. W.; Herman, G. G.; Goeminne, A. M. *Polyhedron* **1999**, *18*, 1029. (d) Carvalho, S.; Delgado, R.; Drew, M. G. B.; Félix, V.; Figueira, M.; Henriques, R. T. *Polyhedron* **2008**, *27*, 679.
- (a) Carvalho, S.; Delgado, R.; Drew, M. G. B.; Félix, V. *Dalton Trans.* **2007**, 2431. (b) Motekaitis, R. J.; Martell, A. E.; Lecomte, J.-P.; Lehn, J.-M. *Inorg. Chem.* **1983**, *22*, 609.
- (a) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831. (b) Nolan, E. M.; Jaworski, J.; Okamoto, K.-I.; Hayashi, Y.; Sheng, M.; Lippard, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 16812. (c) Swamy, K. M. K.; Kim, H. N.; Soh, J. H.; Kim, Y.; Kim, S.-J.; Yoon, J. *Chem. Commun.* **2009**, 1234.
- (a) O'Neil, E. J.; Smith, B. D.; *Coord. Chem. Rev.* **2006**, *250*, 3068. (b) Hargrove, A. E.; Nieto, S.; Zhang, T.; Sessler, J. L.; Anslyn, E. V. *Chem. Rev.* **2011**, *111*, 6603. (c) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23.
- (a) Ojida, A.; Park, S.-K.; Mito-oka, Y.; Hamachi, I.; *Tetrahedron Lett.* **2002**, *43*, 6193. (b) Ojida, A.; Mito-oka, Y.; Inoue, M.-A.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256. (c) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454. (d) Jang, H. H.; Yi, Sujung, Kim, M. H.; Kim, S.; Lee, N. H.; Han, M. S. *Tetrahedron Lett.* **2009**, *50*, 6241.
- de Silva, S. A.; Zavaleta, A.; Baron, D. E.; Allam, O.; Isidor, E. V.; Kashimura, N.; Percarpio, J. M. *Tetrahedron Lett.* **1997**, *38*, 2237.